Table 2. Dissociation constants (K_d) of PTD4-ATF for the target and mutated DNA probes

	DNA probe	K_d	
Name	**Sequence (5' -3')**	**nM**	**K_d/ K_d (WT)**
WT	GGGGCTGGGGGCGGTGTCT	1.08	1
Mut-1	GGGCCTGGGGGCGGTGTCT	7.65	7
Mut-2	GGGGCTGCGGGCGGTGTCT	8.53	8
Mut-3	GGGGCTGGGGGTGGTGTCT	5.25	5
Mut-4	GGGGCTGGGGGCGGTCTCT	4.68	4
Mut-5	CAGGCTGGGGGCGGTGTCT	4.02	4
Mut-6	AGGGCTGGGGGCGGTGTCA	1.91	2
Mut-7	GGGCCTGCGGGCGGTGTCT	38.70	36
Mut-8	GGGCCTGCGGGTGGTGTCT	16.80	16
Mut-9	GGGCCTGCGGGTGGTCTCT	846.00	783

Synthetic DNA duplexes consisted of the sequence 5'-TATATATA(N19)TATATATA-3'. Underlined letters indicate the mutations. Apparent K_d are determined by titration using an electrophoretic mobility shift assay (EMSA) as described below. Each value reported represents the mean of at least two independent experiments. EMSA binding reactions were performed in a final volume of 10 μl using a 3'-end biotin-labeled oligonucleotide (0.2 fmol per reaction) and various amounts of purified designed regulatory proteins (DRPs) in binding buffer: 10 mM Tris·HCl, pH 7.5/100 mM NaCl/1 mM $MgCl_2$/0.1 mM $ZnCl_2$/0.02% BSA/1 μg of poly(dA-dT)$_2$/10% glycerol. Binding reactions were carried out at 4ºC for 30 min. The samples were loaded on a 6% polyacrylamide (acrylamide/bisacrylamide = 33:1) nondenaturing gel (45 mM Tris-borate) and electrophoresed in 0.5× TB buffer at 15 V/ cm for 2 h at 4ºC. The gel was blotted and then visualized by using LightShift Chemiluminescent kit (Pierce) according to the manufacture's instructions. The bands were quantified by using IMAGEQUANT software. Experimental data were fitted to the appropriate equation by using KALEIDAGRAPH software (version 3.0).